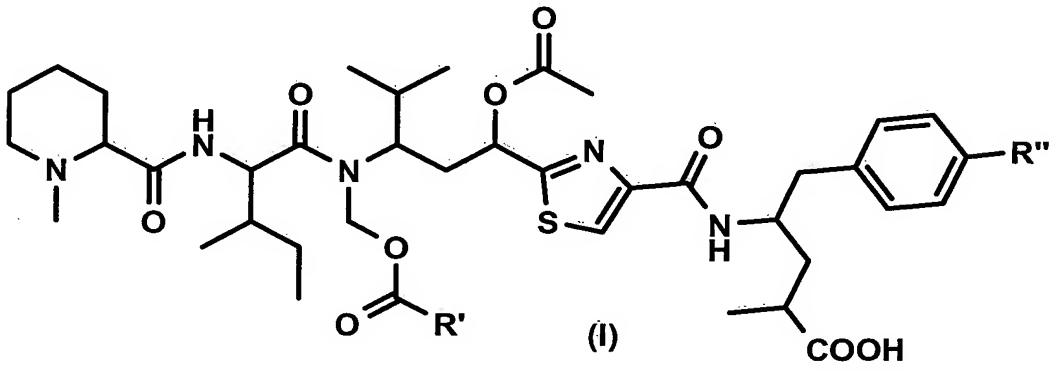
New Tubulysin Analogs

The present invention refers to novel tubulysin analogs and its use for the treatment of cancer diseases.

Tubulysins, for the first time were isolated by Höfle and Reichenbach et al. (GBF Braunschweig) from a culture browth of the myxobacterial strains of *Archangium gephyra* (F. Sasse et al. J. Antibiot. 2000, 53, 879-885; WO9813375; DE 10008089). These compounds show high cytotoxicity in the low picomolare IC_{50} in a panel of cancer cell lines; thus they are of interest as potential anticancer therapeutics. Tubulysins (I) are tetrapeptides, containing three unusual amino acids; thus the total synthesis pose a considerable challenge to organic chemists.



(I)

Tubulysin A: R′ = $CH_2CH(CH_3)_2$; R′′ = OH

Tubulysin B: R′ = $CH_2CH_2CH_3$; R′′ = OH

Tubulysin C: R′ = CH_2CH_3; R′′ = OH

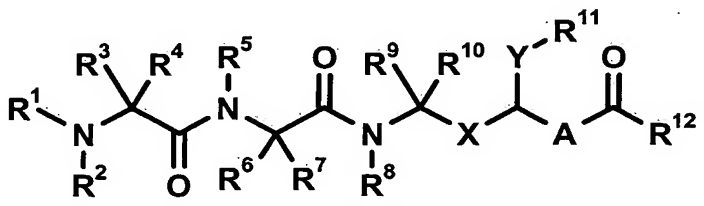
Tubulysin D: R′ = $CH_2CH(CH_3)_2$; R′′ = H

Tubulysin E: R′ = $CH_2CH_2CH_3$; R′′ = H

Tubulysin F: R' = CH_2CH_3; R'' = H

It is an objective of the present invention to provide novel Tubulysin analogues with improved activity and properties, in particular pharmacological properties as compared to the natural products.

The present invention provides a compound of Formula (II):



(II)

wherein

A is a substituted 5- or 6-membered heteroaryl;

wherein

X is O, S or NR^{13} or $CR^{14}R^{15}$;

wherein

Y is O, S or NR^{16};

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16} are independently H, alkyl, alkenyl, alkinyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl, or two R's are members of a cycloalkyl or heterocycloalkyl ring system;

wherein compounds of Formula (I) are excluded,

wherein R' are H, alkyl, alkenyl, aryl or heteroaryl and - at the same time - R" are H, -OH, alkyl, aryl, or heteroaryl;

or a pharmacologically acceptable salt, a solvate, a hydrate or a pharmacologically acceptable formulation thereof. Explicitely excluded are Tubulysins A, B, C, D, E and F.

The term alkyl or alk refers to a saturated, linear or branched hydrocarbon group, containing from one to twenty carbonatoms, preferably from one to twelve carbon atoms, mostly preferred from one to six carbon atoms, for example methyl, ethyl, propyl, isoporpyl, isobutyl, n-butyl, tert-butyl, n-hexyl, 2,2-dimethylbutyl or n-octyl.

The term alkenyl and alkinyl refers to a at least partially unsaturated, linear or branched hydrocarbon group, containing from two to twenty carbonatoms, preferably from two to twelve carbon atoms, mostly preferred from two to six carbon atoms, for example ethenyl, allyl, acetylenyl, prpargyl, isoprenyl, or hex-2-enyl. Preferentially alkenyl groups contain one or two, mostly preferred one double bond and alkinyl group contain one or two, mostly preferred one triple bond.

Optionally the term akyl, alkenyl and alkinyl refers to groups where one or several, preferentially one, two or three hydrogen atoms are replaced by a halogen atom, prferentially fluorine or chlorine or a 2,2,2-trichlorethyl, or a trifluoromethyl.

The term heteroalkyl refers to a alkyl, alkenyl or alkinyl group, where several, preferentially one, two or three carbon atoms are replaced by a O, N, P, B, Se, Si, or S atom, preferentially O, S, N. The term

heteroalkyl refers to a carboxylic acid or a thereof derived group, for example acyl (alkyl-CO), acylalkyl, alkoxycarbonyl, acyloxy, acyloxyalkyl, carboxyalkylamid or alkoxycarbonyloxy.

Examples of heteroalkyl groups are groups of the formula R^a-O-Y^a-, R^a-S-Y^a-, R^a-N(R^b)-Y^a-, R^a-CO-Y^a-, R^a-O-CO-Y^a-, R^a-CO-O-Y^a-, R^a-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-Y^a-, R^a-O-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-O-Y^a-, R^a-N(R^b)-CO-N(R^c)-Y^a-, R^a-O-CO-O-Y^a-, R^a-N(R^b)-C(=NR^d)-N(R^c)-Y^a-, R^a-CS-Y^a-, R^a-O-CS-Y^a-, R^a-CS-O-Y^a-, R^a-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-Y^a-, R^a-O-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-O-Y^a-, R^a-N(R^b)-CS-N(R^c)-Y^a-, R^a-O-CS-O-Y^a-, R^a-S-CO-Y^a-, R^a-CO-S-Y^a-, R^a-S-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-S-Y^a-, R^a-S-CO-O-Y^a-, R^a-O-CO-S-Y^a-, R^a-S-CO-S-Y^a-, R^a-S-CS-Y^a-, R^a-CS-S-Y^a-, R^a-S-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-S-Y^a-, R^a-S-CS-O-Y^a-, R^a-O-CS-S-Y^a-, wherein R^a refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^b refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^c refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^d refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group and Y^a refers to a direct binding, a C_1-C_6-alkylen, a C_2-C_6-alkenylen or a C_2-C_6-alkinylen group, wherein each heteroalkyl group can be replace by a carbon atom and one or several hydrogen atoms can be replaced by fluorine or chlorine atoms. Examples of heteroalkyl groups are methoxy, trifluormethoxy, ethoxy, n-propyloxy, iso-propyloxy, tert-butyloxy, methoxymethyl, ethoxymethyl, methoxyethyl, methylamino, ethylamino, dimethylamino, diethylamino, iso-propylethylamino, methyl-aminomethyl, ethylaminomethyl, di-iso-propylaminoethyl, enolether, dimethylaminomethyl, dimethylaminoethyl, acetyl, propionyl, butyryloxy, acetyloxy, methoxycarbonyl, ethoxy-carbonyl, N-ethyl-N-methylcarbamoyl or N-methylcarbamoyl. Other examples of heteroalkyl groups are nitrile, isonitrile, cyanate, thiocyanate, isocyanate, isothiocyanate and alkylnitrile groups.

The term cycloalkyl refers to a saturated or partially unsaturated (e.g. cycloalkenyl) cyclic group, comprising one or several rings, preferntialy one or two, containing three to fourteen ring carbon atoms, preferentially three to ten, preferentially three, four, five, six or seven ring carbon atoms. Furthermore the term cycloalkyl refers to a group where one or more hydrogen atoms are replaced by F, Cl, Br, I, OH, =O, SH, =S, NH_2, =NH, or NO_2, or cyclic ketones, for example cyclohexanone, 2-cyclohexenone or cyclopentanone. Examples of cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentenyl, spiro[4,5]-decanyl, norbornyl, cyclohexyl, cyclopentenyl, cyclohexadienyl, decalinyl, cubanyl, bicyclo[4.3.0]nonyl, tetralin, cyclopentylcyclohexyl, fluor-cyclohexyl or the cyclohex-2-enyl group.

The term heterocycloalkyl refers to the above definition, wherein a or several, preferentialy one, two or three ring carbon atoms are replaced by a O, N, Si, Se, P, or S, prferentially O, S, N. Preferentially a heterocycloalkyl goups is composed of one or two rings comprising three to ten, preferentially three, four, five, six or seven ring atoms. Moreover the term heterocycloalkyl refers to groups where a or several hydrogen atoms are replaced by F, Cl, Br, I, OH, =O, SH, =S, NH_2, NO_2. Examples of heterocycloalkyl are piperidyl, morpholinyl, urotropinyl, pyrrolidinyl, tetrahydrothiophenyl, tetrahydropyranyl, tetrahydro-furyl, oxacyclopropyl, azacyclopropyl or 2-pyrazolinyl groups as well as lactams, lactons, cyclic imides and cyclic anhydrides.

The term alkylcycloalkyl refers to groups, which contain cycloalkyl as well as alkyl, alkenyl or alkinyl groups according to the above definition, e.g. alkylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkyl and alkinylcycloalkyl groups. Preferentially a alkylcycloalkyl group is composed of a cycloalkyl group, comprising one or more rings, comprising

three to ten, preferentially three, four, five, six or seven carbon - atomes and one or two alkyl, alkenyl oder alkinyl groups with one or two to six carbon atoms.

The term heteroalkylcycloalkyl refers to alkylcycloalkyl groups, according to the above definition, wherein one or several, preferentialy one, two or three carbon atoms are replaced by O, N, Si, Se, P or S, preferentialy O, S, N. Preferentially it is composed of a heteroakylcycloalkyl group comprising one or two ring systems with three to ten, preeferentialy three, four, five, six or seven ring atoms and one or two alkyl, alkenyl, alkinyl or heteroalkyl groups with one or two to six carbon atoms. Examples of such a group are alkylheterocycloalkyl, alkylheterocycloalkenyl, alkenyl-heterocycloalkyl, alkinylheterocycloalkyl, heteroalkyl-cycloalkyl, heteroalkylheterocycloalkyl and heteroalkylheterocylcloalkenyl, wherein the cyclic group is saturated or partially (simply, twofold or threefold) unsaturated.

The term aryl or ar refers to a aromatic group, composed of one or several rings, comprising six to fourteen carbon atoms, preferentially six to ten, prferentially six carbon atoms. The term aryl or ar refers to a aromatic group, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. Examples are phenyl-, naphthyl-, piphenyl-, 2-fluorphenyl, anilinyl-, 3-nitrophenyl or 4-hydroxy-phenyl.

The term heteroaryl refers to a aromatic group, composed of one or several rings, comprising five to fourteen rind atoms, preferentially five to ten, and a or several, preferentially one, two, three or four O, N, P or S ring atoms, prferentially O, S or N. The term heteroaryl refers to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. Examples are 4-pyridyl, 2-imidazolyl, 3-

phenylpyrrolyl, thiazolyl, oxazolyl, triazolyl, tetrazolyl, isoxazolyl, indazolyl, indolyl, benzimidazolyl, pyridazinyl, chinolinyl, purinyl, carbazolyl, acridinyl, pyrimidyl, 2,3´-bifuryl, 3-pyrazolyl and isochinolinyl.

The term aralkyl refers to groups, in accordance to the above definition, composed of aryl and alkyl, alkenyl, alkinyl and/or cycloalkyl, e.g. arylalkyl, arylalkenyl, arylalkinyl, arylcycloalkyl, arylcycloalkenyl, alkylarylacycloalkyl and alkylarylcycloalkenyl. Examples of aralkyles are toluol, xylol, mesitylen, styren, benzylchloride, o-fluortoluene, 1H-inden, tetralin, dihydronaphthaline, indanon, phenylcyclopentyl, cumol, cyclo-hexylphenyl, fluoren and indan. Preferentially, a aralkyl group is composed of composed of one or two aromatic rings, comprising six to ten ring carbon atoms and one or two alkyl, alkenyl and/or alkinyl comprising one or two to six carbon atoms and/or one cyclo-alkyl comprising five or six ring carbon atoms.

The term heteroaralkyl refers to groups, in accordance to the above definition, wherein one or several, preferentially one, two, three or four carbon atoms are replaced by O, N, Si, Se, P, B, S, preferentially O, N or S, and groups which according to the above definition contain aryl, heteroaryl and alkyl, alkenyl, alkinyl and/or heteroalkyl and/or cycloalkyl cnd/or heterocyclo-alkyl. Preferentially a heteroaralkyl group is composed od a or two aromatic ring systemes comprising five or six to ten carbon atoms and one or two alkyl, alkenyl and/or alkinyl comprising one or two to six carbon atoms and/or one cycloalkyl comprising five or six ring carbon atoms, wherein one, two, three or four carbon atoms can be replaced by O, N or S.

Examples are arylheteroalkyl, arylheterocycloalkyl, aryl-heterocycloalkenyl, arylalkylheterocycloalkyl, aryl-alkenylheterocycloalkyl, arylalkinylheterocyclo-alkyl, arylalkylheterocycloalkenyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkinyl, heteroarylheteroalkyl, heteroarylcycloalkyl, heteroarylcycloalkenyl, heteroarylheterocycloalkyl, hetero-arylheterocycloalken-yl, heteroarylalkylcycloalkyl, heteroarylalkylhetero-cycloalkenyl, heteroarylheteroalkylcycloalkyl, Hetero-arylheteroalkylcycloalkenyl and heteroarylheteroalkyl heterocycloalkyl, wherin the cyclic groups can be saturated or simple, twice, three fold of four fold unsaturated. Examples are tetrahydroiso-chinolinyl, benzoyl, 2- or 3-ethyl-indolyl, 4-methylpyridino, 2-, 3- or 4-methoxyphenyl, 4-ethoxyphenyl, 2-, 3- or 4-carboxyphenylalkyl.

The terms cycloalkyl, heterocycloalkyl, alkylcyclo-alkyl, heteroalkylcycloalkyl, aryl, heteroaryl, aralkyl and heteroaralkyl refer to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2.

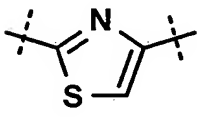
The term "optinally substituiert" relates to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. The term "gegebenenfalls substituiert" relates further to groups, comprising exclusively or in addition unsubstituted C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkinyl, C_1-C_6 heteroalkyl, C_3-C_{10} cycloalkyl, C_2-C_9 heterocycloalkyl, C_6-C_{10} aryl, C_1-C_9 heteroaryl, C_7-C_{12} aralkyl or C_2-C_{11} heteroaralkyl.

Protecting groups are known to the specialist and described in P. J. Kocienski, Protecting Groups, Georg Thieme Verlag, Stuttgart, 1994 and in T. W. Greene, P. G. M. Wuts, Protective Groups in Organic Synthesis, John

Wiley & Sons, New York, 1999. Common amino protecting groups are e.g. *t*-butyloxycarbonyl (Boc), benzyloxycarbonyl (Cbz, Z), benzyl (Bn), benzoyl (Bz), fluorenylmethyloxycarbonyl (Fmoc), allyloxycarbonyl (Alloc), trichlorethyloxycarbonyl (Troc), acetyl or trifluoracetyl.

Compounds of Formula (II) can comprise several chiral centers related to their substitution pattern. The present invention relates to all al defined enantio and diastereo isomers as well as their mixtures in all ratios. Moreover the present invention relates to all cis/trans isomers of compounds of the general Formula (II) as well as thier mixtures. Moreover the present invention relates to all tautomeric forms of compounds of the general Formula (II).

Preferably A constitutes a optinally substituted thizol ring; more preferably A has the following structure:



Moreover preferably X constitutes a CH_2 group.

Preferably Y constutites O.

Preferably R^1 constitutes a C_1-C_4 alkyl.

Preferably R^2 ans R^3 constitute together $(CH_2)_n$ with n = 2, 3, 4 or 5.

Preferably R^4 constitutes H or methyl.

Preferably R^5 constitutes H.

Preferably R^5 constitutes C_1-C_6 alkyl, C_3-C_6 cycloalkyl or C_4-C_7 lkylcycloalkyl.

Preferably R^5 constitutes H or methyl.

Preferably R^8 constitutes CH_2OCOR^{17}, wherein R^{17} constitutes C_1-C_6 alkyl or C_1-C_6 alkenyl.

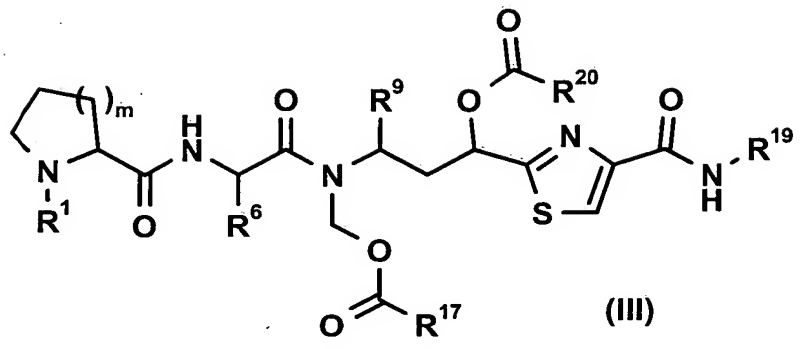
Preferably R^9 constitutes C_1-C_6 alkyl.

Preferably R^{10} constitutes H or methyl.

Preferably R^{11} constitutes H or $-(C=O)-(C_{1-4})$Alkyl.

Preferably R^{12} constitutes $NR^{18}R^{19}$, wherein R^{18} constitutes H or methyl and R^{19} constitutes aralkyl or heteroaralkyl.

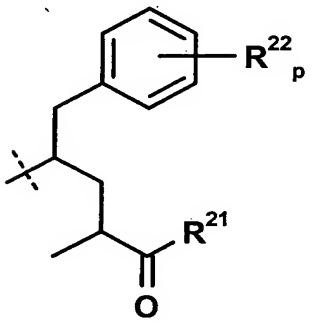
Most preferably are compounds of Formula (III),



(III)

wherein R^1 comprise C_1-C_4 alkyl, R^6 comprise C_1-C_6 alkyl, R^9 comprise C_1-C_6 alkyl, R^{17} comprise C_1-C_6 alkyl or C_1-C_6 alkenyl, R^{19} comprise aralkyl or heteroaralkyl, R^{20} comprise C_1-C_4 alkyl and m equals 1 or 2.

Preferentially R^{19} comprise the following structure:



wherein R^{21} comprise OH, NH_2, alkyloxy, alkyl amino or dialkyl amino, R^{22} comprise halogen, OH, NO_2, NH_2, alkyloxy, alkyl amino or dialkyl amino and p equals 0, 1, 2 or 3.

Examples of pharmacologically acceptable salts of compounds of Formula (II) are physiologically acceptable mineral acids, e.g. hydrochloric acid, sulfuric acid, phorphoric acid or salts of organic acids, e.g. methansulfonic acid, p-toluenesulfonic acid, lactic acid, formic acid, trifluoracetic acid, citric acid, succinic acid, fumaric acid, maleic

acid and salicylic acid. Compounds of Formula (II) can be solvated, especially hydrated. The hydration can occur during the synthesis process or can be a consequence of the hygroscopic nature of the originally dehydrated compound of Formula (II). Compounds of Formula (II), containing assymetric carbon atoms might exist as mixtures of diastereomers, as mixtures of enantiomers or as optically pure compounds.

The pharmaceutical composition according to the present invention is composed of at least one compound of Formuly (II) and optinally carrier and/or adjuvants.

Pro drugs are also subject of the present invention and they are composed of a compound of Formula (II) and at least one pharmakologically acceptable protecting group, which is cleaved under physilogical conditions, e.g. alkoxy, aralkyloxy, acyl or acyloxy, more precicely ethoxy, benzyloxy, acetyl or acetyloxy. Moreover the present invention relates to conjugates comprising at least one compound of Formula (II) and a biological macromolecule, e.g. oligo saccharide, monoclonale antibody, lectine, PSA (prostata specific antigen) or peptidic vectors and if needed as well as a suitable linker. The expression linker relates to a chemical group, which links compounds of Formula (II) with a biological macromolecule. Examples of linkers are alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl.

The therapeutic usage of compounds of Formula (II), its pharmacologic acceptable salts and/or its solvates and hydrates, as well as the corresponding formulations and pharmacological compositions are also subject of the present invention.

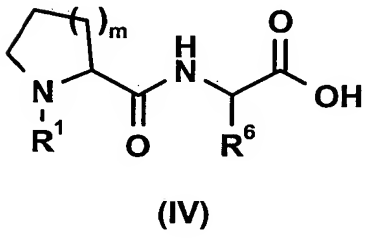
The usage of the active agents for the preperation of drugs for the treatment of cancer is also subject of the present invention. Moreover the present compounds are of interest for the prevention and/or treatment of rheumatoid arthritis, inflammatory diseases, immunological diseases (e.g. type I diabetis), autoimmune diseases, other tumor diseases as well as for the surface treatment (impregnation) of plastic and metal implants, e.g. stents. In general, compounds of Formula (II) will be given as a single treatment or in combination with an arbitrary therapeutic substance according to known and accepted modes. Such therapeutically useful compositions can be administered in one of the following ways: orally, including dragees, coated tablets, pills, semi-solids, soft or hard capsules, solutions, emulsions or suspensions; parenteral, including injectable solutions; rectal as suppositories; by inhalation, including powder formulation or as a spray, transdermal or intranasal. For the production of such tablets, pills, semi solids, coated tabletts, dragees and hard gelatine capsules the therapeutically used product is mixed with pharmacologically inert, anorganic or organic carriers, e.g. with lactose, sucrose, glucose, gelatine, malt, silical gel, starch, or derivatives thereof, talkum, stearinic acid or its salts, dried skim milk and the like.

For the production of soft capsuls a carrier one may use for example vegetable oils, petroleum, animal or synthetic oils, wax, fat, polyols. For the production of liquide solutions and syrups one may use carriers for example water, alcohols, aqueous saline, aqueous dextrose, polyole, glycerin, vegatable oils, petroleum, animal or synthetic oils. For the production of suppositories one may use excipients as are e.g. vegetable, petroleum, animal or synthetic oils, wax, fat and polyols. For aerosol formulations one may use compressed gases suitable fort his purpose,as are e.g. oxigen, nitrogen, noble gas and carbon dioxide. The pharmaceutically useful agents may also contain additives for conservation, stabilisation, e.g. UV stabilizer, emulsifier, sweetener,

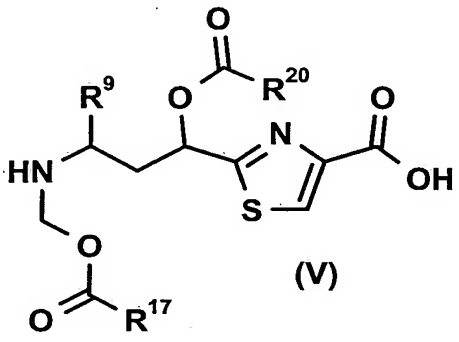
aromatiser, salts to change the osmotic pressure, buffers, coating additives and antioxidants.

Combinations with other therapeutic agents can include further agents, which are commonly used to treat cancer.

Compounds of Formula (IV), (V) and (VI) provided with suitable protecting groups are produced as building blocks for the of compounds of Formula (II). These can be linked *via* peptide coupling methods using known coupling reagents, e.g. hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC) or dicyclohexylcarbodiimide (DCC).



(IV)

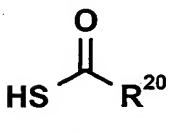


(V)

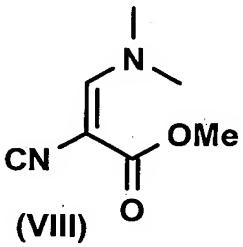
(VI)

Building block (IV) can be assembled through peptide coupling of commercially availbable and known aminoacids.

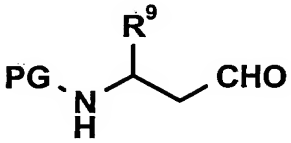
Building block (V) can be assembled through a muticomponent reaction of starting materials of Formula (VII), (VIII) und (IX).



(VII)



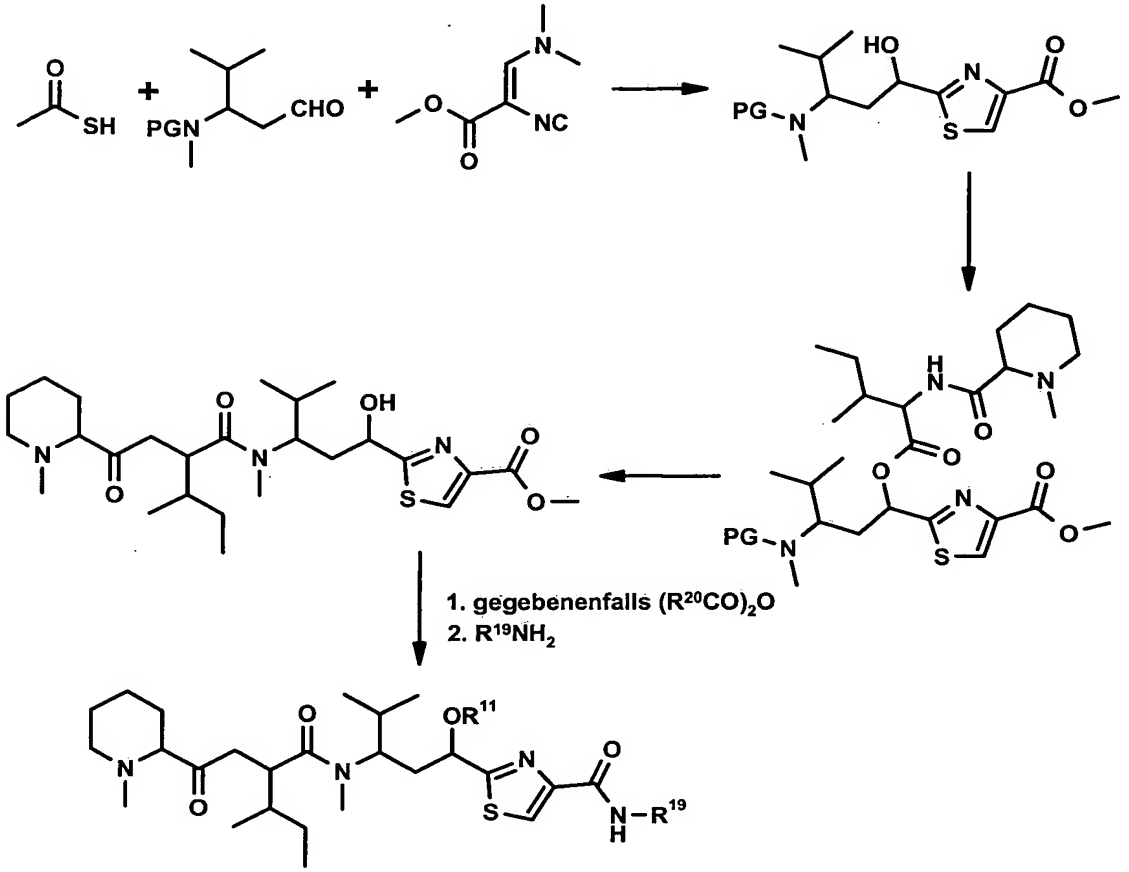
(VIII)



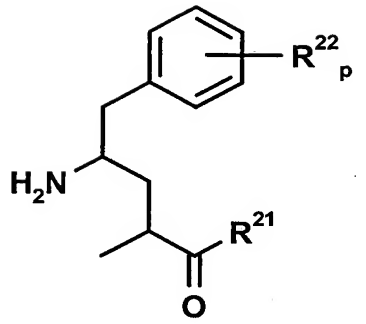
(IX)

Herein PG is a known amino protecting group, for example tert-butyloxycarbonyl (Boc). The resulting compound can be further transformed to building block (V) using $R^{17}COOCH_2Cl$ or H_2CO and $R^{17}COOH$ or H_2CO, TMS-Cl and $R^{17}COONa$ (I. Kornonen et al. Acta Chem. Scand. Ser. B 1982, 36(7), 467-474; R. Moriera et al. Tetrahedron Lett. 1994, 35(38), 7107-7110; R. W. A. Luke, Tetrahedron Lett. 1996, 37(2), 263-266).

Alternatively compounds of Formula (III) can be synthesized according to the following scheme:

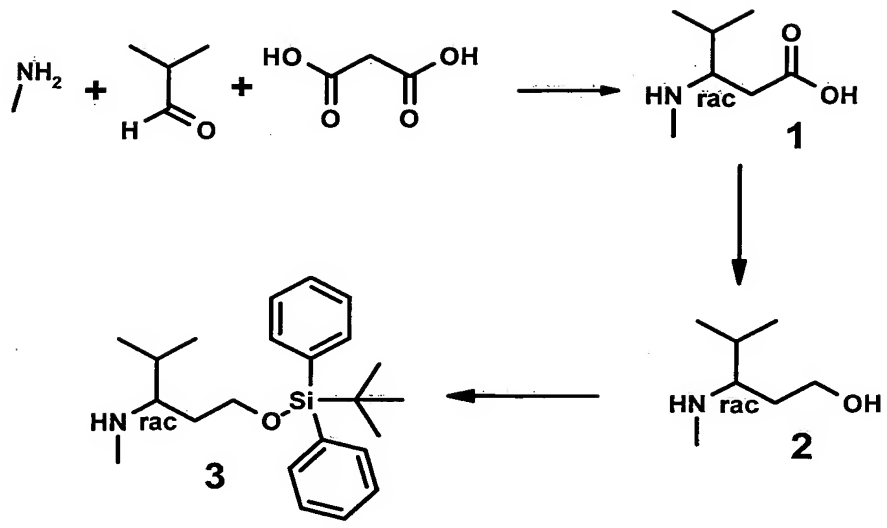


Building block (VI) of the following Formula:



can be stereoselectively synthesized using Evens reaction.

Examples



Synthesis of N-methyl-β-R,S-valine (1)

58.8 ml (0.47mol) of a 8M methylamine solution in ethanol are slowly dropped to a solution of 33.8g isobutyric aldehyde (0.47mol) in 200ml ethanol while keeping the temperature in the flask below 5°C. Then 50ml THF are added and the mixture is refluxed for 1h. Then 48.91g (0.47mol) malonic acid is added in small portions and the mixture is refluxed for 5h. After cooling to 25°C the prcipitated is filtered off, washed with THF and dried under high vaccuum. Yield: 50.34g N-methyl-□-R,S-valine.

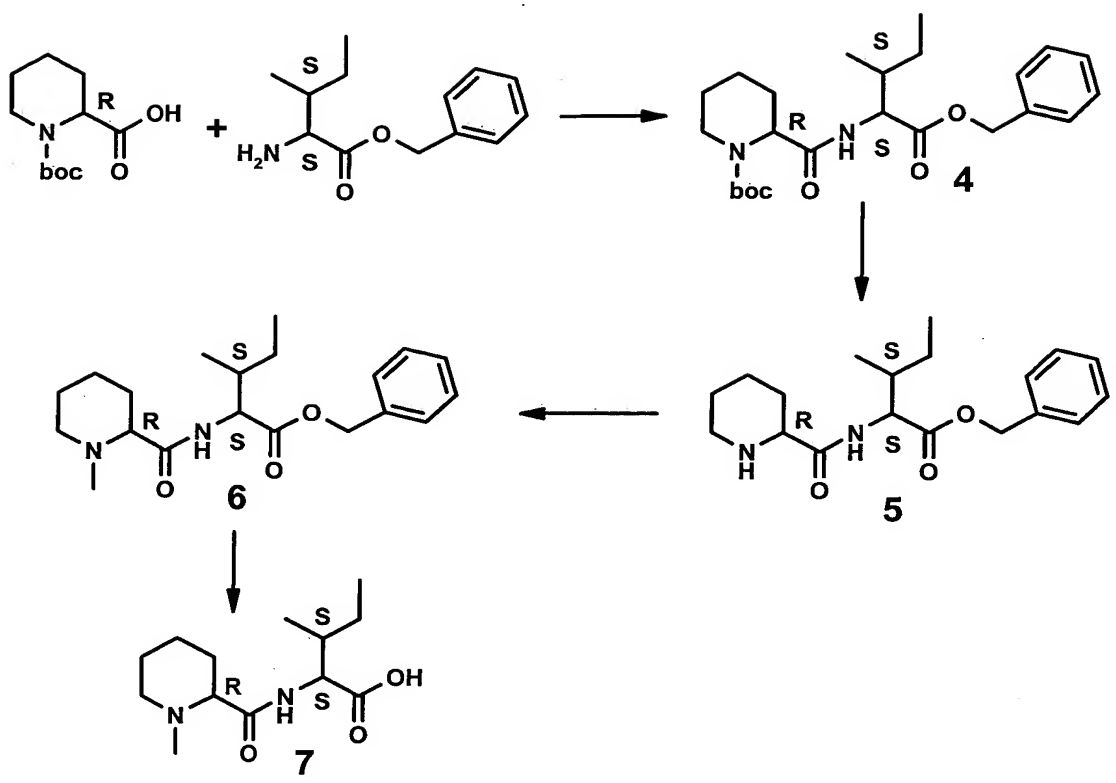
Mass spectroscopy: expected molecular mass 145.2; found: m/z $(M+H)^+$ = 146.1.

Synthese von N-Methyl-β-R,S-valinol (2)

14.5g (0.1mol) N-methyl-β-R,S-valine in 135 ml dry THF are added slowly to 150ml 1M lithiumaluminium hydrid in THF (0.15mol) while keeping the temperature in the flask below 5°C. This mixture is refluxed for 4h. Subseqently the mixture is stirred over night. The mixture is hydrolized with 4ml 12% KOH and 4ml water. The precipitate is filtered off and is extracted two times with 80ml hot THF. The filtrates are combined and the solvent is removed under vaccuum. The resulting oil is destillled (bp.: 48°C/0.5mbar). Yield: 8.28g N-methyl-β-R,S-valinol. Mass spectroscopy: expected molecular mass 131.2; found: m/z $(M+H)^+$ = 132.2.

Synthesis of N-methyl-β-R,S-valinolyl-tert.-butyldiphenyl-silylether (3)

2g N-Methyl-β-R,S-valinole (15.24mmol) are solubilized in 20ml dry dichlormethan together with 465.5mg dimethylaminopyridin (3.81mmol) and 2.66ml triethylamine (19.05mmol). To this solution 4.61ml tert.-butyldiphenylsilylchloride (18mmol) is added and the mixture is stirred over night. 20ml Water and 20ml dichlormethane are added. The water phase is extracted two times with dichlormethane and the combined organic phases are dried over sodium sulfat. The sodium sulfate is filtered of and the solvent is evaporated undeer vaccuum. The residual oil is purified using column chromatography (eluent: ethylacetat/ethanol = 8:2). Yield: 3.94g N-nethyl-β-R,S-valinolyl-tert.butyldiphenylsilylether. Mass spectroscopy: expected molecular mass 369.6; found: m/z $(M+H)^+$ = 370.5.



Assembly of the dipeptide (R)-N-Boc-homoPro-(S,S)-Ile-OBzl (4)

7g 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (21.81mmol) and 2.4ml N-methylmorpholin (21.81mmol) are added to a solution of 5g (R)-N-Boc-homoprolin (21.81mmol) in 40ml dry DMF. After 10 minutes 7.21g (S,S)-H-Ile-OBzl tosylat (18.32mmol) and 2ml N-methylmorpholin (18.32mmol) are added. This mixture is stirred over night at 25°C and then 40ml ethylacetate are added. The organic layer is washed with saturated $NaHCO_3$. The aqueous layer is extracted two times with ethylacetate. The combined organic extracts are washed with saturated NaCl and dried over Na_2SO_4. The solvent is evaporated under vaccuum and the pure product appears. Yield 5.54g (R)-N-Boc-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 432.6; found: m/z $(M+H)^+$ = 433.6

Boc-deprotection of (R)-N-Boc-homoPro-(S,S)-Ile-OBzl (5)

To a solution of (R)-N-Boc-HomoPro-(S,S)-Ile-OBzl in 60ml dry THF is added 120ml 4M HCl in dioxan while keeping the temperature in the flask

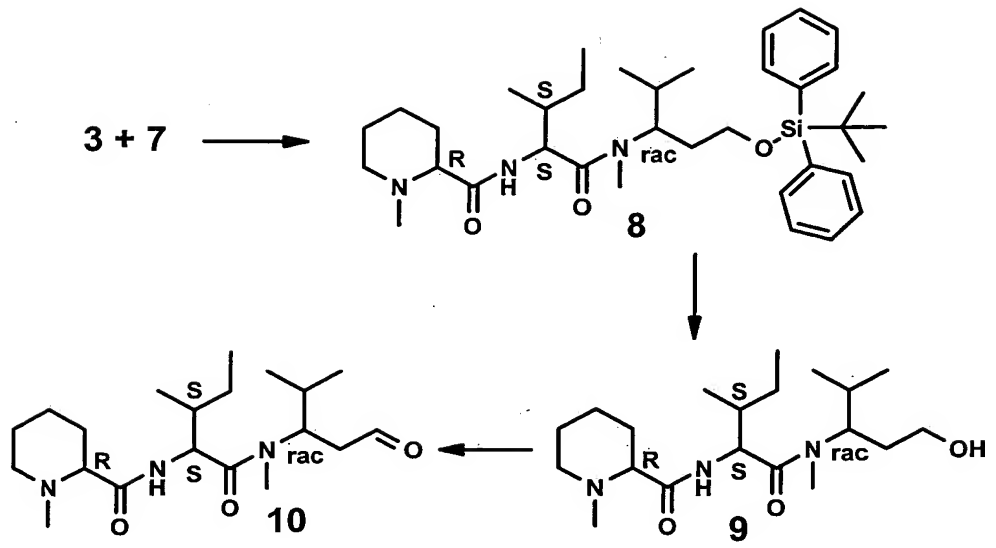
below 5°C. After allowing the temperature to come to 20°C the mixture is stirred for 5 h. The solvent is evaporated and can be used directly without further purification for the next step. Yield: 4.1g (R)-H-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 332.5; found: m/z $(M+H)^+$ = 333.6.

Reductive amination of (R)-H-homoPro-(S,S)-Ile-OBzl (6)

10ml of a 37% formaldehyde solution (123mmol) is added to 4.1g (R)-homoPro-(S,S)-Ile-OBzl (12.3mmol) in 20ml methanol. The pH is addjusted to 5-6 ewith acetic acid and 1.932g sodium cyanoborhydride (30.75mmol) is added in portions. The mixture is stirred for 16h at 20°C. Subsequently the reaction is acidified with conc. HCl. The solvent is evaporated under vaccuum and water is added. The pH is addjusted to pH 12 with solide NaOH and the mixture is extracted three times with dichlormethan. The organic layer is dried with Na_2SO_4 and the solvent is evaporated. The resulting oil is evaporated by column chrommatography (eluent: ethylacetat : n-heptan = 1:1). Yield: 3.9g (R)-N-methyl-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 346.5; found: m/z $(M+H)^+$ = 347.4

Hydration of (R)-N-methyl-homoPro-(S,S)-Ile-OBzl (7)

To a solution of 3.9g (R)-N-methyl-homoPro-(S,S)-Ile-OBzl (11.26mmol) in 30ml methanol, 1.2g Pd (10%C) are added. The flask is first flushed with N_2 and then 10 min with H_2. Two more h the suspension is stirred under a H2-ballone; then the catalyst is filtered through celite, and washed two times with methanol. The solvent is evaporated and the residual oil is lyophylized giving a white powder. Yield: 2.7g (R)-N-methyl-homoPro-(S,S)-Ile-OH. Mass spectroscopy: expected molecular mass 256.4; found: m/z $(M+H)^+$ = 257.4



Coupling of (R)-N-methyl-homoPro-(S,S)-Ile-OH and N-methyl-β-R,S-valinolyl-tert.butyldiphenylsilylether (8)

To a solution of 3.522g (R)-N-methyl-homoPro-(S,S)-Ile-OH (13.74mmol) in 15ml dry DMF, 2.104g hydoxybenzotriazol (13.74mmol) and 2.151ml diisopropylcarbodiimide (13.74mmol) are added. After 15 minutes stirring 4.232g N-methyl-β-R,S-valinolyl-tert.butyldiphenylsilylether (11.45mmol) is added and the mixture is stirred for 16h at 20°C. The precipitated diisopropyl urea is filtered off and the solvent is evaporated under vaccuum. The residue is thoroughly stirred wit dichlomethane and the residual diisopropyl urea is filtered off. The dichlormethan solutionis extracted with NaHCO3 and dryed subsequently with Na2SO4. After filtering off the Na2SO4 the solvent is evaporated under vaccuum. The residue is purified with preperative HPLC. (RP-C18, eluent methanol + 0.5%acetic acid / water + 0.5% acetic acid). Yield: 3.91g. Mass spectroscopy: expected molecular mass 608.0; found: m/z $(M+H)^+$ = 609.0.

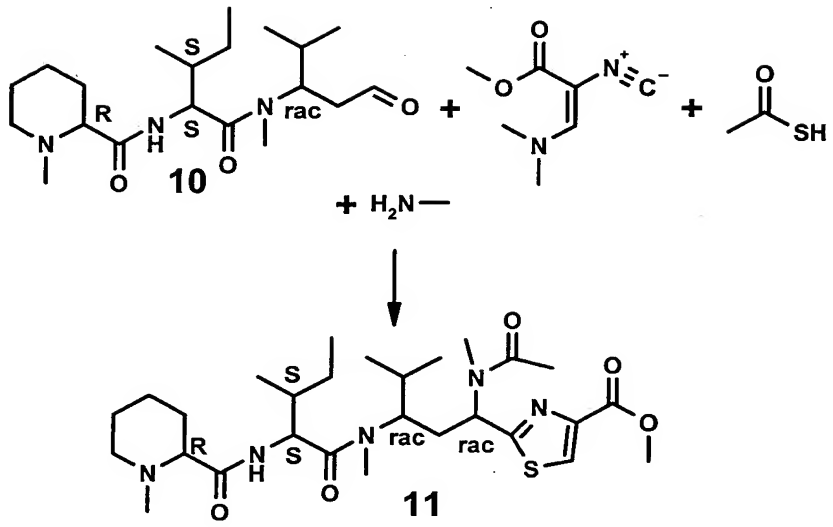
Deprotection of the tert.butyldiphenylsilyl protecting group of (8) (9)

3.91g Of compound 8 (6.43mmol) are solubilized in 30ml dry tetrahydrofuran and 2.223ml tetrabutylammoniumfluorid (1M in THF) (7.72mmol) are added dropwise and the resulting mixture is stirred for 2h at 20°C. Then 8 ml of water is added and the tetrahydrofuran is

evaporated under vaccuum. The solution is neutralized and extracted five times with ethylacetat. The combined organic phases are extracted two times with saturated NaCl and dried over Na_2SO_4. The Na_2SO_4 is filtered off and the solvent is evaporated. The resulting product is pure enough for further transfromations. Mass spectroscopy: expected molecular mass 369.6; found: m/z $(M+H)^+$ = 370.5.

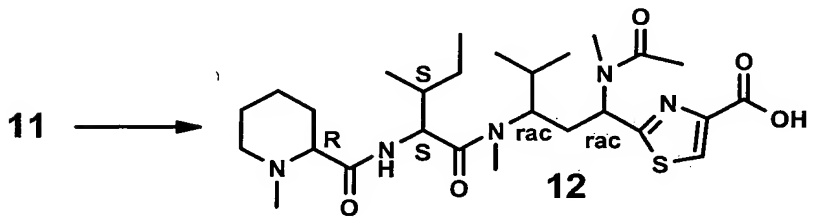
Swern-Oxidation of (9) (10)

A solution of 0.665ml oxalylchloride (7.75mmol) in 25ml dry dichlormethan in a 250 ml flask is cooled to -70°C under a N_2 atmosphere. Slowly 1.188ml dimethylsulfoxide (16.73mmol) in 5ml dry dichlormethane is added in a way that the inner temperature is kept below -60°C and the resulting mixture is stirred for 30 minutes at -70°C. Then a solution (6ml) of (9) (6.43mmol) in dichlormethane is added in a way that the inner temperature is kept below -60°C. After stirring for further 30 minutes 4.459ml triethylamin (32.17mmol) are added at -70°C. Once the flask reached 20°C, 15ml water are added and further 10 minutes are stirred. The aqueous phase is extracted two times with dichlormethan. The combined orgaic phases are dryed over Na_2SO_4, the Na_2SO_4 is filtered off and the solvent is evaporated. The resulting product is pure enough to be used in the next step. Mass spectroscopy: expected molecular mass 367.6; found: m/z $(M+H)^+$ = 368.5.



Thiazolsynthesis (11)

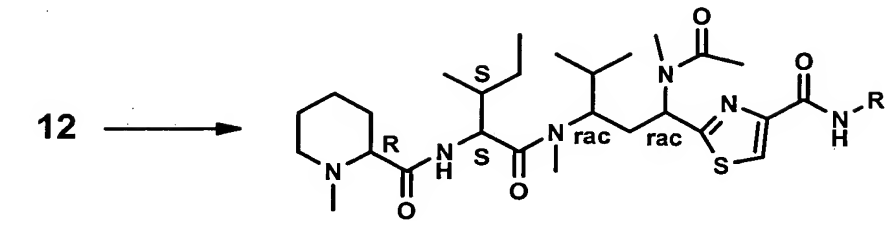
0.695ml Methylamin solution (33% in ethanol) (7.72mmol) are added to (10) in 20ml dry methanol and stirred for 1h at 20°C. 991.3mg 3-Dimethylamino-2-isocyano-acrylacidmethylester (6.43mmol) and 0.457ml thioacetic acid (6.43mmol) are added and stirred for 16h at 20°C. The slvent is evaporate under vaccuum and the residue is purified by preperative HPLC (reversed phase-C18-phase, eluent methanol + 0.5% acetic acid / water + 0.5% acetic acid). Yield: 1.294g. Mass spectroscopy: expected molecular mass 565.8; found: m/z $(M+H)^+$ = 566.7

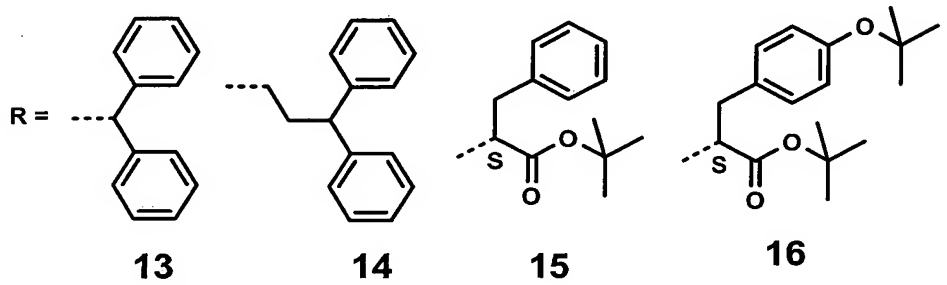


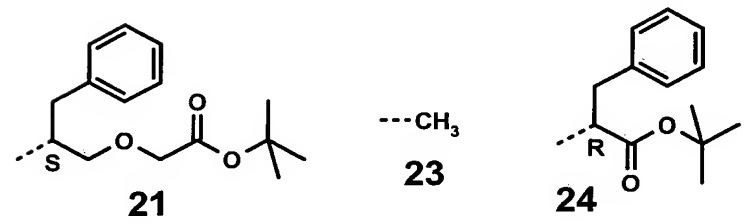
Saponification (11) (12)

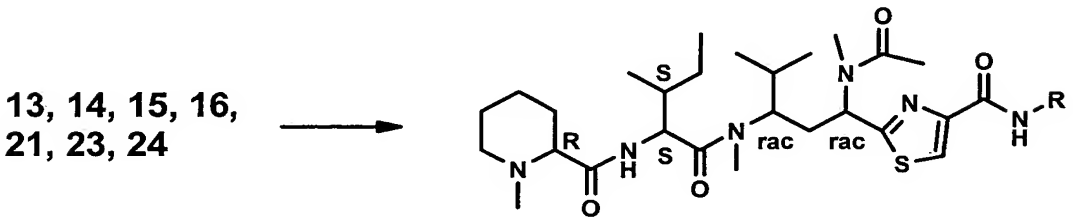
To a solution of 1.294g (11) (2.29mmol) in 20ml THF 220mg LiOH (9.16mmol) in 20ml wate rare added and stired for 16h at 20°C. This mixture is neutralized with 2N HCl. The solvent is evaporated under reduced pressure and the residue is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield:

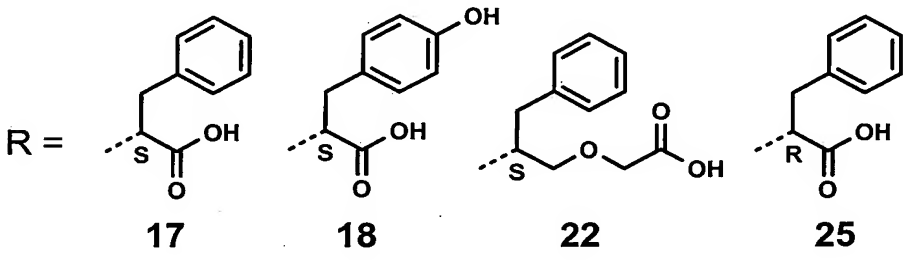
1.14g. Mass spectroscopy: expected molecular mass 551.8; found: m/z $(M+H)^+$ = 552.7











Coupling of (12) and □-aminodiphenylmethane (13)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 0.062ml α-aminodiphenylmethan (0.36mmol) are added. This mixture is

stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 717.0; found: m/z $(M+H)^+$ = 718.1

Coupling of (12) and 3,3-diphenylpropylamine (14)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 76mg 3,3-diphenylpropylamin (0.36mmol) are added. This mixture is stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 745.0; found: m/z $(M+H)^+$ = 746.1.

Coupling of (12) and S-phenylalanine tert.butylester (15)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 24.3mg S-phenylalanine tert.butylester (0.11mmol) are added. This mixture is stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 755.0; found: m/z $(M+H)^+$ = 756.2.

Coupling of (12) and S-tyrosin-O-tert.-butylether-tert.-butylester (16)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 32.3mg S-tyrosin-O-tert.-butylether-tert.-butylester (0.11mmol) are

added. This mixture is stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 827.1; found: m/z $(M+H)^+$ = 828.0.

Deprotection of (15) (17)

To a solution of 26mg (15) (0.034mmol) 2ml dry dichlormethan 2ml trifluoracetic acid are added. The mixture is stirred for 1h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 20mg. Mass spectroscopy: expected molecular mass 698.9; found: m/z $(M+H)^+$ = 699.5.

Deprotection of (16) (17)

To a solution of 26mg (16) (0.034mmol) 2ml dry dichlormethan 2ml trifluor aceticacid are added. The mixture is stirred for 1h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 18mg. Mass spectroscopy: expected molecular mass 714.9; found: m/z $(M+H)^+$ = 715.5.

Coupling of benzyloxycarbonyl-S-phenylalaninol and bromo aceticacid-tert.-butyl-ester (19)

To a solution of 1.141g benzyloxycarbonyl-S-phenylalaninol (4mmol) in 20ml dry THF 160mg sodiumhydrid dispersion (60% in mineral oel) are added. After end of H_2 evolution 1.182ml bromo acetic acid tert.-butylester (8mmol) are addedand the mixture is stirred for 48h at 20°C. The solvent is evaporated under reduced pressure and the product is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetci acid). Yield: 805mg. Mass spectroscopy: expected molecular mass 399.5; found: m/z $(M+H)^+$ = 400.3

Cbz-deprotection of (19) (20)

To a solution of 805mg (19) (2.02mmol) in 15ml methanol, 800mg Pd (10%C) are added. The flask is first flushed with N_2 and then stirred 16 h under H2 atmosphere (2 H2 ballons). The catalyst is filtered through celite and washe several times with methanol. The solvent is evaporated. Yield: 482mg. Mass spectroscopy: expected molecular mass 265.4; found: m/z $(M+H)^+$ = 266.3.

Coupling of (12) and (20) (21)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 16.8mg hydroxybenzotriazol hydrate (0.11mmol) and 0.014ml diisopropylcarbodiimid (0.11mmol) are added. After stirring for 15 minutes at 20°C 29.2mg (20) (0.11mmol) are added. After stirring over night at 20°C the solution is filtered and the residue is pruified by HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid / water+0.5% acetic acid). Yield: 22mg. Mass spectroscopy: expected molecular mass 799.1; found: m/z $(M+H)^+$ = 800.2.

Deprotection of (21) (22)

To a solution of 22mg (21) (0.028mmol) in 2ml dry dichlormethan 2ml trifluoracetic acid are added. This mixture is stirred for 1 h at 20°C and the solvent is evaporated upon addition of n-heptan. The product is pure. Yield: 16mg. Mass spectroscopy: expected molecular mass 757.0; found: m/z $(M+H)^+$ = 758.2.

Coupling of (12) and methylamin (23)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 0.22ml methylamin solution (2M in THF) (0.44mmol) are added. This mixture is stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic

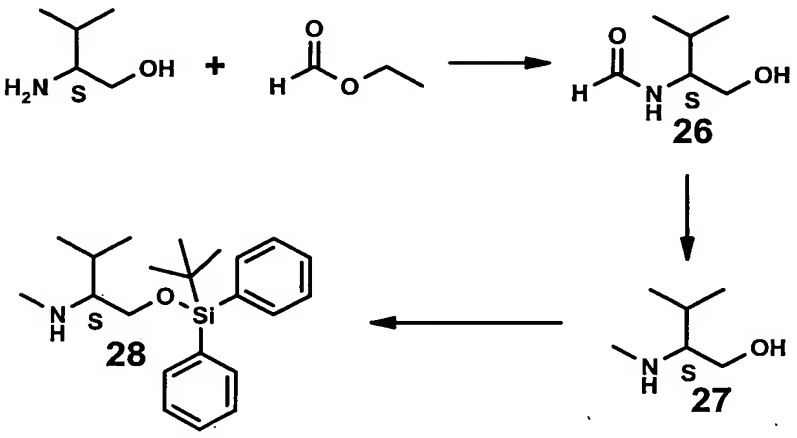
acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 564.8; found: m/z $(M+H)^+$ = 565.7.

Coupling of (12) and R-Phenylalanintert.butylester (24)

To a solution of 49.5mg (12) (0.09mmol) in 3ml dry DMF 18.6mg 6-chlorhydroxybenzotriazole (0.11mmol) and 0.014ml diisopropylcarbodiimide (0.11mmol) are added. This mixture is stirred for 15 minutes at 20°C and 24.3mg R-phenylalanine tert.butylester (0.11mmol) are added. This mixture is stirred over night at 20°C, then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 35mg. Mass spectroscopy: expected molecular mass 755.0; found: m/z $(M+H)^+$ = 756.2.

Deprotection of (24) (25)

To a solution of 23mg (24) (0.03mmol) in 2ml dry dichlormethan 2ml trifluoracetic acid are added. The mixture is stirred for 1h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 18mg. Mass spectroscopy: expected molecular mass 698.9; found: m/z $(M+H)^+$ = 699.5.



Synthesis of N-formyl-S-valinol (26)

10g S-Valinol (97mmol) are dissolved in 50ml ethylformiat and refluxed for 1h. The solvvent is evaporated and the rsidue is destilled under

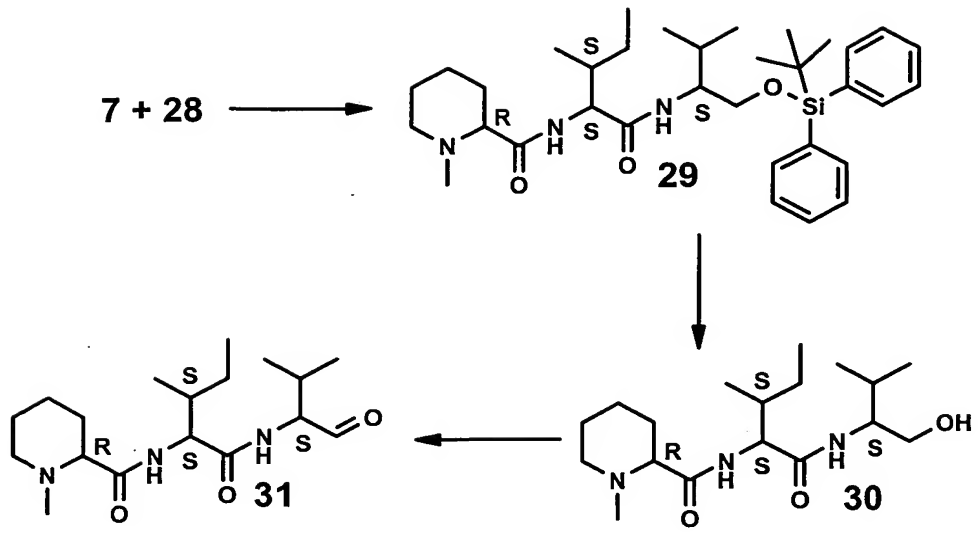
vacuum (bp.: 153°C/0.5mbar). Yield: 8.4g. Mass spectroscopy: expected molecular mass 131.2; found: m/z $(M+H)^+$ = 132.3

Synthesis of N-methyl-S-valinol (27)

To a solution of 5.7g lithiumaluminiumhydrid (150mmol) in 200ml dry THF, 8.4g N-formyl-S-valinol (64mmol) dissolved in 40 ml dry THF are added slowly and stirred for 16h at 20°C. In several portions 30g sodium sulfat decahydrat and 18ml water are added and furthermore stirred for 3h at 20°C. The solids are filtered off and the solvent is evapüorated under vaccuum. The residual material is purified by destillation (bp.: 93°C/54mbar). Yield: 3.7g. Mass spectroscopy: expected molecular mass 117.2 found: m/z $(M+H)^+$ = 118.1.

Synthesis of N-methyl-S-valinolyl-tert.butyldiphenylether (28)

To a solution of 1.64g N-methyl-S-valinol (14mmol) in 10ml dry dichlormethane 427mg dimethylaminopyridine (3.5mmol) and 2.44ml triethylamin (17.5mmol) are added. Then 4.3ml tert.butyldiphenylsilyl chloride are added and 16h stirred at 20°C. Then 10ml water and THF are added and the phases are seperated. The aqueous phase id extracted two times with dichlormethane. The combined organic phases are dried ove Na_2SO_4, subsequently the solvent is evaporated. The residue is purified by column chromatography (eluent: ethylacetat/ethanol = 8:2). Yield: 3.16g. Mass spectroscopy: expected molecular mass 355.6 found: m/z $(M+H)^+$ = 366.6



Coupling of (R)-N-methyl-homoPro-(S,S)-Ile-OH and N-methyl-S-valinolyl-tert.butyldiphenylsilylether (29)

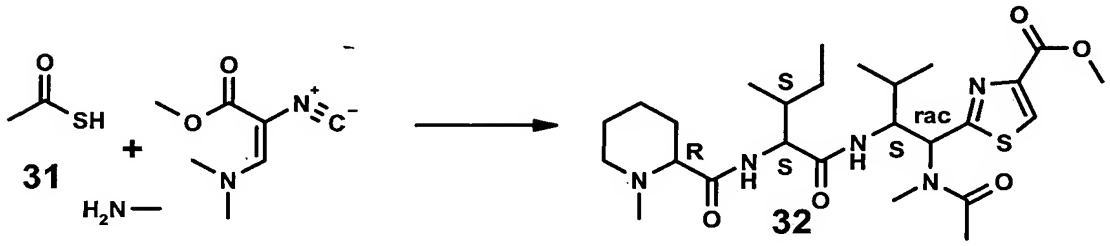
To a solution of 1.54g (R)-N-methyl-homoPro-(S,S)-Ile-OH (6mmol) in 10ml dry DMF, 1.02g 6-chlorohydroxybenzotriazol (6mmol) and 0.939ml diisopropylcarbodiimid (6mmol) are added. The mixture is stirred for 15 minutes and 2.56g N-ethyl-S-valinolyl-tert.butyldiphenylether (7.2mmol) are added and stirred for 16h at 20°C. Then the solvent is evaporated ubder vacuum and the residue is purified by preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid / water+0.5% acetic acid). Yield: 1.06g. Mass spectroscopy: expected molecular mass 593.9 found: m/z $(M+H)^+$ = 594.8.

Cleavage of the tert.-butyldiphenylsilyl protecting group of (29) (30)

To a solution of 1.06g (29) (1.79mmol) in 10ml dry THF a solution of 2.15ml tetrabutylammoniumfluorid (1M Lösung in THF) (2.15mmol) is added. The mixture is stirred for 16h at 20°C and then hydrolysed upon addition of 3ml water. The organic solvent is evaporated and the aqueous phase is extracted five times with ethylacetat. The combined organic phases are washed with staturated NaCl and dried over Na_2SO_4. After filtration of Na_2SO_4 the solvent is evaporated. Yield: 1.05g (some residual silyl is remaining). Mass spectroscopy: expected molecular mass 355.5 found: m/z $(M+H)^+$ = 356.5.

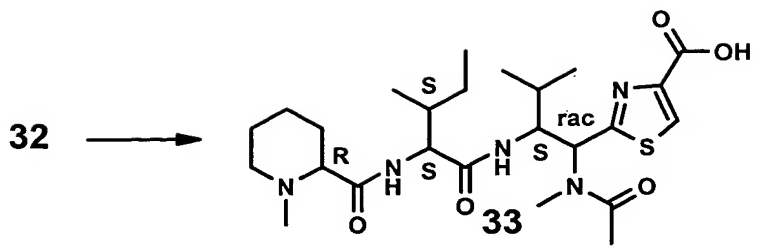
Swern-Oxidation of (30) (31)

0.316ml Oxalylchlorid (1.98mmol) are solubilized in 3ml dry dichlormethan in a 100ml flask under N_2 atmosphere and cooled to -70°C. To this solution 0.305ml dimethylsulfoxid (4.29mmol) in 0.6ml dichlormethan are added slowly (evolution of gas, keep the temperatur below -60°C) and stirring continues ofr 30 minutes. A solution of 587mg (30) (1.65mmol) in 2ml dichlormethan is added while keeping the temperature below -60°C and stirring for 30 minutes. Then 1.146ml triethylamin (8.25mmol) is added. The mixture is allowed to come to 20°C and then 10ml water are added and the mixture is stirred for another 10 minutes. The aqueous phase is extracted two times with dichlormethan. The combined organic layers are dried with Na_2SO_4. After filtering off the Na_2SO_4 the solvent is evaporated. Yield: 636mg. Mass spectroscopy: expected molecular mass 353.5 found: m/z $(M+H)^+$ = 354.5.



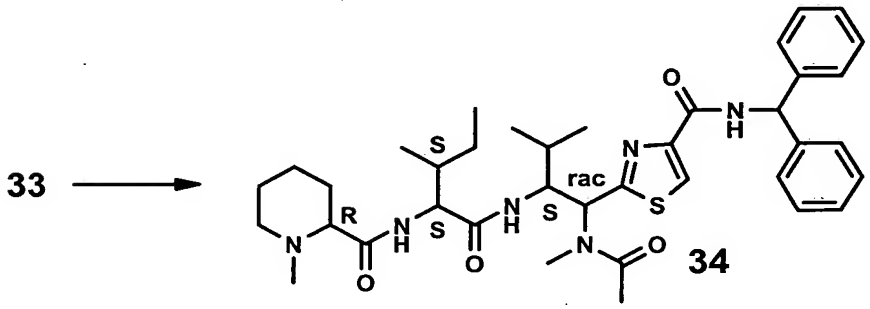
Thiazolsynthesis (32)

636 Mg (31) (1.15mmol) and 0.173ml methylamin (33% in ethanol) (1.38mmol) in 3ml dry methanol are stirred for 1h at 20°C. 185mg 3-Dimethylamino-2-isocyano-acrylciacidmethylester (1.2mmol) and 0.086ml thioacetic acid (1.2mmol) are added and stirred for 16h at 20°C. The solvent is evaporated and the residue is purified with preperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 150mg. Mass spectroscopy: expected molecular mass 551.8; found: m/z $(M+H)^+$ = 552.7.



Saponification of (32) (33)

To a solution of 61g (32) (0.11mmol) in 2ml THF, 10.6mg LiOH (0.44mmol) in 2ml water is added and stirred for 16h at 20°C. The mixture is neutralized with 2N HCl. The solvent is evaporated and the residue is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid). Yield: 50mg. Mass spectroscopy: expected molecular mass 537.7; found: m/z $(M+H)^+$ = 538.7.



Coupling of (33) and α-aminodiphenylmethane (34)

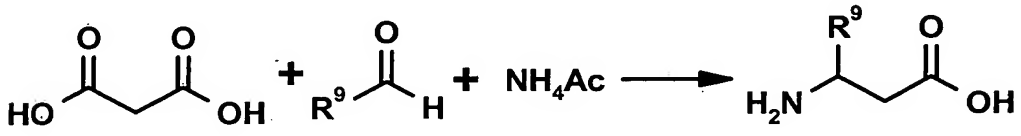
To a solution of 49.5mg (33) (0.093mmol) in 3ml dry DMF, 14.2mg hydroxybenzotriazol (0.093mmol) and 0.012ml diisopropylcarbodiimid (0.093mmol) are added and stirred for 15 minutes at 20°C. 0.064ml α-aminodiphenylmethan (0.372mmol) is added and is stirred over night. The mixture is filtered and evaporated and the residue is purified by preparative HPLC (reversed phase-C18-phase, eluent methanol +0.5%acetic acid / water+0.5% acetic acid). Yield: 30mg. Mass spectroscopy: expected molecular mass 703.0; found: m/z $(M+H)^+$ = 704.1.

General procedure for the synthesis of thiazoles:

1 Mmol of the carbonyl compound (IX) is solubilized in 3 ml dry THF gelöst under N_2 atmosphere and 1 mmol borontrifluorid etherat are added.

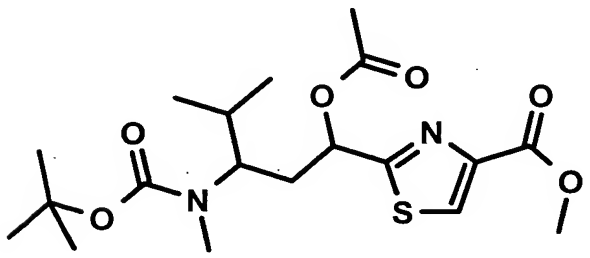
After 10 min 1 mmol of isocyanide (VIII) and 1 mmol of thioacarboxylic acid (VII) are added and stirred for 72h. Water is added and optinally filtered through celite. The solvent is evaporated under vacuum. The residue is solubilized in ethylacetate. The organic phase is washed two times with water. After drying the organic phase over Na_2SO_4 the slvent is evaporated. The residue is purified by preperative HPLC (reversed phase-C18-phase, eluent methanol+0.5%acetic acid / water+0.5% acetic acid).

Compounds of Formula (IX) can be synthesized for example by a α-aminoalkylation of isobutyric aldehyd, ammoniumacetat or a primary amine or amine hydrochlorid and malonic acid:



The resulting β-amino acid can be subsequently N-alkylated (e.g. by reductive amination) and protected (e.g. t-butyloxycarbonyl, Boc). Then the carboxylic acid group is transformed to the aldehyde (e.g. by reduction to the alkohol by $LiAlH_4$ and subsequent Swern oxidation to the aldehyde; see for example R. C. Larock, Comprehensive Organic Transformations, VCH Publishers, New York, 1989). Alternatively the β-aminoacid can be synthesized by a Arndt-Eistert procedure starting from valine.

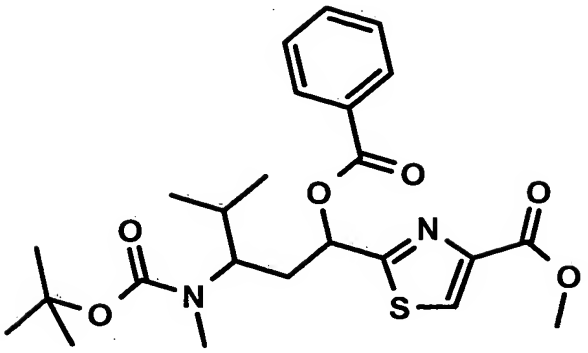
Example 35:



$C_{19}H_{30}N_2O_6S$ (414.5248)

MS (ESI): 415 [M+H]

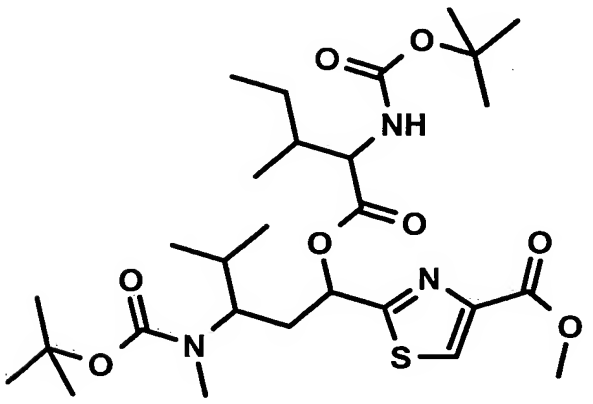
Example 36:



$C_{24}H_{32}N_2O_6S$ (476.5964)

MS (ESI): 477 [M+H]

Example 37:

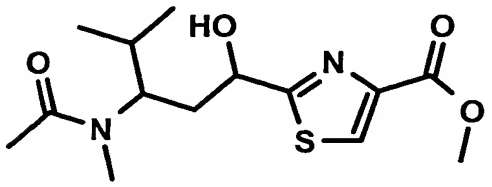


$C_{28}H_{47}N_3O_8S$ (585.7661)

MS (ESI): 586 [M+H]

Example 38:

A compound from example 35 (0.1 mmol) is stirred in 2 ml dichlormethan (DCM) and 0.1 ml trifluoracetic acid (TFA) for 1 h at 20°C. The liquides DCM/TFA are evaporated and the residue is purified by HPLC.

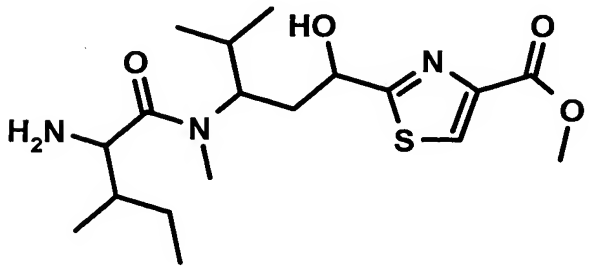


$C_{14}H_{22}N_2O_4S$ (314.4064)

MS (ESI): 315 [M+H]

Example 39:

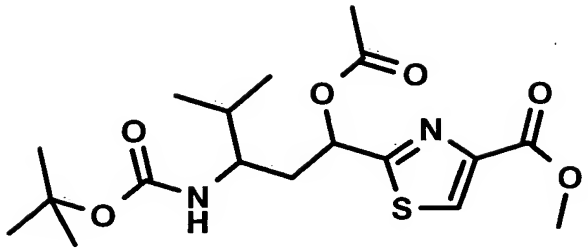
The compound from example 37 (0.1 mmol) is dissolved in 2 ml Ddchlormethan (DCM) and 0.1 ml trifluoracetic acid (TFA) is added and stirred for 1 h at 20°C. The liquides DCM/TFA are evaporated and the residue is purified by HPLC.



$C_{18}H_{31}N_3O_4S$ (385.5295)

MS (ESI): 386 [M+H]

Beispiel 40:

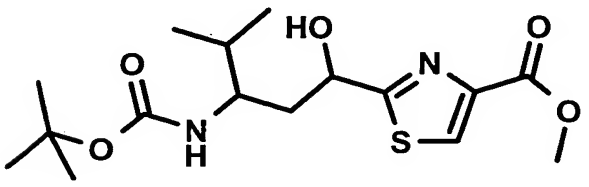


$C_{18}H_{28}N_2O_6S$ (400.4977)

MS (ESI): 401 [M+H]

Example 41:

1 mmol of the compound from example 40 in 1 ml methanol is stirred with 1 ml 4 M ammonia solution in methanol for 2h at 20°C. Tsolvent is evaporated under vacuum.



$C_{16}H_{26}N_2O_5S$ (358.4600)

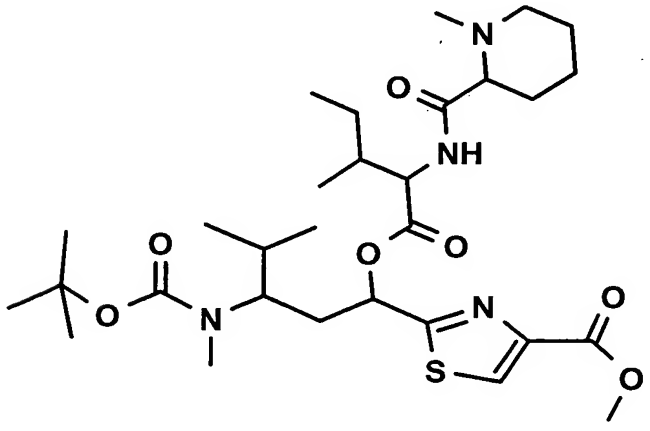
MS (ESI): 381 [M+Na]

Example 42 and 43:

Ester coupling of hydroxythiazols (example 41) and dipeptide (7) and subsequent transacylation:

To 2 Mmol (512 mg) 3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoic acid (7) in 5 ml dry dichlormethan is added 2 mmol (252 mg) *N,N'*-diisopropylcarbodiimide (DIC) in 2.5 ml DCM and 0,2 mmol (24 mg) DMAP in 2.5 ml DCM under N_2 atmosphere at 0°C. The mixture is stirred 5 minutes at 0°C. 1 mmol (372 mg) 2-[3-(*tert.*-butoxycarbonyl-methyl-amino)-1-hydroxy-4-methyl-pentyl]-thiazole-4-carboxylic acid methylester (example 41) is dissolved in 5 ml DCM and slowly added via syringe. The mixture is stirred 4 h at 20°C. The mixture concentrated in vacuum and the precipitated urea is filtered off. To the filtrate is added 1 ml of trifluoracetic acid and 1 h stirred at 20°C and the solvents are evaporated under vacuum. The residue is dissolved in 1 ml dry dichlormethan and 1 ml triethylamin is added and 1 h stirred at 20°C. The solvent is evaporated under vacuum. The rearranged coupling product is purified by HPLC.

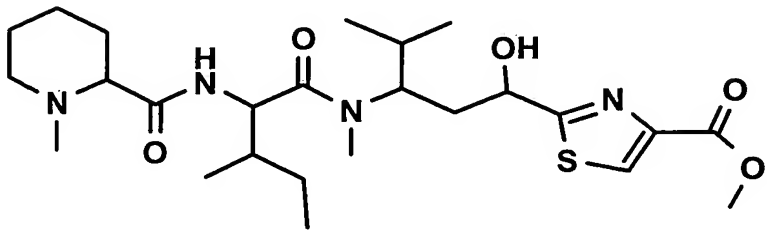
2-(3-(*tert.*-butoxycarbonyl-methyl-amino)-4-methyl-1-{3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoyloxy}-pentyl)-thiazol-4-carboxylic acid methylester (42):



$C_{30}H_{50}N_4O_7S$ (610,82)

MS (ESI): 611 [M+H]; 633 [M+Na]

2-[1-Hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazol-4-carboxylic acid methylester (43):



$C_{25}H_{42}N_4O_5S$ (510,70)

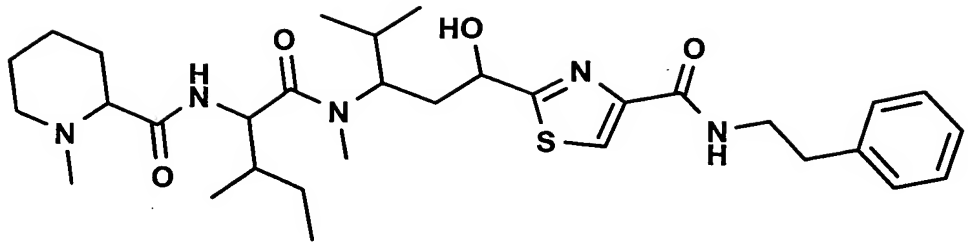
MS (ESI): 511 [M+H]; 533 [M+Na]

Example 44 and 45:

Reaction of (43) and phenylethylamine and subsequent acetylation

0,14 Mmol (72 mg) 2-[1-hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazol-4-carboxylic acid methylester (43) are stirred with 100 µl phenylethylamine for 12 h at 20°C. The reaction mixture is filtered thhrough a plug of silica gel and washed with ethylacetate. The mixture is evaporated to dryness and 40 µl acetic acid anhydride and 10 µl pyridine are added. The mixture is stirred for 2 h at 20°C. A third of the reaction mixture is purified with a analytical HPLC.

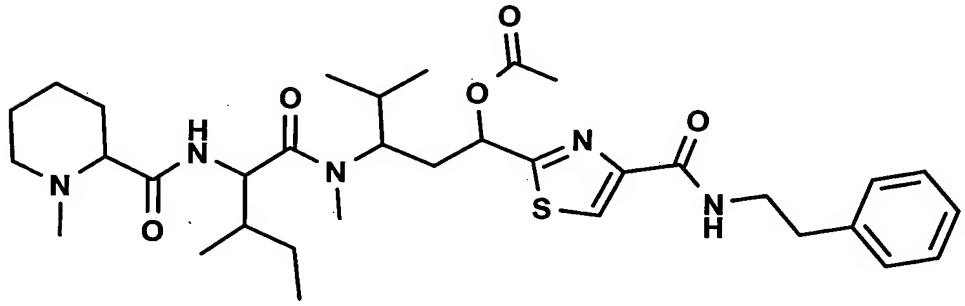
1-methyl-piperidin-2-carbonsäure-[1-({1-[2-hydroxy-2-(4-phenethylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-methyl-carbamoyl)-2-methyl-butyl]-amide (44)



$C_{32}H_{549}N_5O_4S$ (599,84)

MS (ESI): 600 [M+H]; 622 [M+Na]

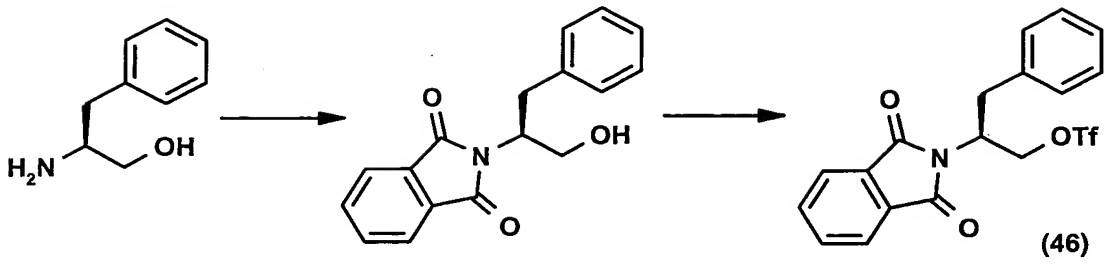
Acetic acid 4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-1-(4-phenethylcarbamoyl-thiazol-2-yl)-pentylester (45)

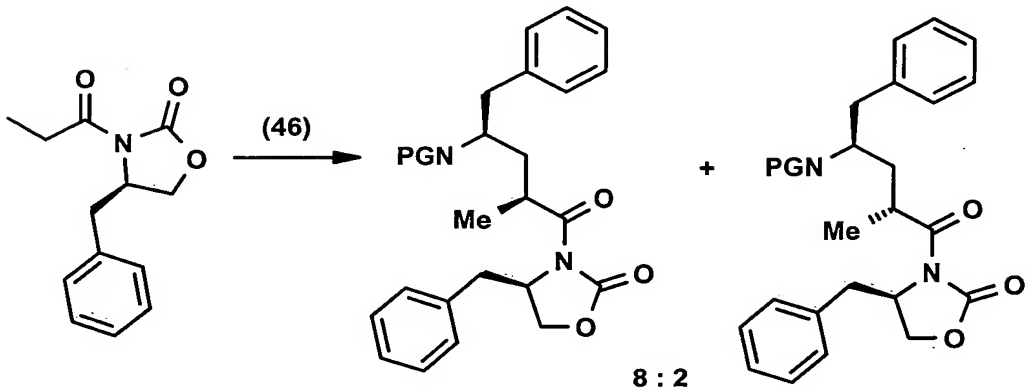


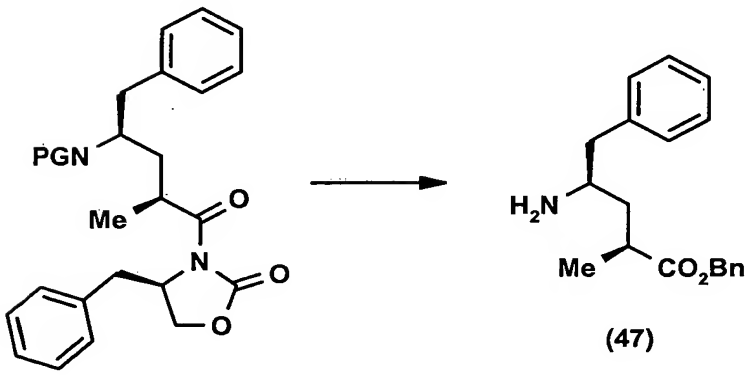
$C_{34}H_{51}N_5O_5S$ (641,88)

MS (ESI): 642 [M+H]; 664 [M+Na]

Synthesis of building block (VI) according to *Evans*-procedure:







(2*S*)-2-Phthalimido-3-phenyl-propanol:

To *L*-phenylalaninol (1.0 g, 6.61 mmol) and Na_2CO_3 (1.05 g, 9.92 mmol) in a 1:1 mixture of THF (10 mL) and H_2O (10 mL) *N*-carbethoxyphthalimide (1.74 g, 7.94 mmol) is added and stirred 4 h at 20°C. To this reaction mixture ethylacetate (20 mL) is added. The aqueous phase is extracted two times with 15 mL ethylacetate and the combined organic phases are washed with saturated NaCl, dried with Na_2SO_4 and the solvent is evaporated under

vacuum. The product is purified with column chromatography using 2% MeOH in CH_2Cl_2. Yield: 1.41 g (76%); MS (ESI) 282 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.76 (m, 2H), 7.73-7.66 (m, 2H), 7.24-7.12 (m, 5H), 4.70-4.58 (m, 1H), 4.12-4.02 (m, 1H), 3.98-3.88 (m, 1H), 3.20 (d, J = 12.5 Hz, 2H), 2.80-2.72 (m, 1H).

(2*S*)-1-Trifluoromethanesulfonyl-2-phthalimido-3-phenyl propanoat:

To a solution of (2*S*)-2-phthalimido-3-phenylpropanole (0.42g, 1.49 mmol) in dry CH_2Cl_2 (5 mL), pyridin (146 µL, 1.79 mmol) is added at -78 °C and stirred for 20 minutes. To this mixture 3 min trifluoromethansulfonic acid anhydrid (264 µL, 1.57 mmol) is added inbetween 3 minutes and stirred for 1 h at -78°C. The reaction mixture is quentched with 3 ml saturated NaCl. The aqueous phase is extracted with 5 mL of CH_2Cl_2, the combined organic phases are washed with 5 ml saturated NaCl gewaschen, dried with Na_2SO_4 and the solvent is evaporated. The product is purified with column chromatography using 20% ethylacetat in hexen. Yield: 0.41 g (66%). MS (ESI) 414 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84-7.77 (m, 2H), 7.75-7.68 (m, 2H), 7.28-7.14 (m, 5H), 5.18 (t, J = 13.0 Hz, 1H), 5.00-4.85 (m, 1H), 4.55-4.30 (m, 1H), 3.40-3.25 (m, 2H).

Evans alkylation:

(4*R*)-3-propanoyl-4-benzyl-2-oxazolidinone (0.100 g, 0.43 mmol) is dissolved in 2 ml dry THF in an argon atmosphere and subsequently cooled to -40°C. LiHMDS (1M/THF) (0.47 mL, 0.47 mmol) is added and stirred for 45 minutes. (2*S*)-1-Trifluoromethansulfonyl-2-phthalimido-3-phenylpropanoate (0.266 g, 0.64 mmol) in dry THF (2 mL) is added. The mixture is stirred for 4h at -40°C and subsequently quentched with 3 ml saturated NaCl. The aqueous phase is extracted 2 times with 5 ml ethylacetate. The combined organic phasesare washed with 3 ml saturated

NaCl, dried with Na_2SO_4 and the solvent is evaporated under vacuum. The product is purified with column chromatograpgy using 25% ethylacetate in hexen. Yield: 0.149 g (70%). The diastereomers can be separated using preperative TLC. The wanted diastereomer is formed in excess: 8:2.

(2'S, 4'R, 4R,)-3-(2'Methyl-4'phthalimido-5'phenyl pentanoyl)-4-benzyl-1,3-oxazolidin-2-one (major product):

MS (ESI): 497 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (t, J = 8.5 Hz, 2H), 7.63 (t, J = 8.4 Hz, 1H), 7.55 (t, J = 8.4 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.37-7.22 (m, 6H), 7.10 (d, J= 8.6 Hz, 2H), 5.08 (q, J = 9.6 and 16.1 Hz, 1H), 4.56-4.42 (m, 2H), 4.20-4.00 (m, 4H), 3.45 (dd, J = 10.7 and 16.1 Hz, 1H), 3.12-2.98 (m, 2H), 2.34 (dd, J = 12.8 and 13.9 Hz, 1H), 1.62 (d, J = 8.6 Hz, 3H).

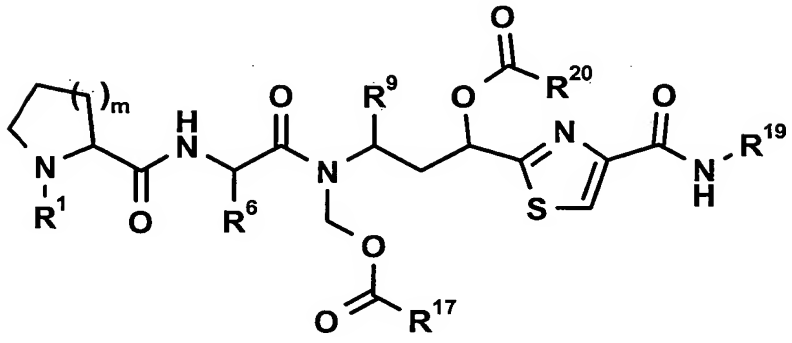
(2'R, 4'R, 4R,)-3-(2'Methyl-4'phthalimido-5'phenyl pentanoyl)-4-benzyl-1,3-oxazolidin-2-one (minor product):

MS (ESI): 497 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, J = 8.6 Hz, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.63 (t, J = 8.6 Hz, 1H), 7.53 (t, J = 8.5 Hz, 1H), 7.40-7.20 (m, 10H), 5.10 (q, J = 7.5 and 15.0 Hz, 1H), 4.94-4.84 (m, 1H), 4.54-4.42 (m, 1H), 4.36-4.08 (m, 4H), 3.46-3.30 (m, 2H), 3.12 (dd, J = 9.6 and 11.8 Hz, 1H), 2.88 (dd, J = 9.5 and 12.8 Hz, 1H), 1.00 (d, J = 9.6 Hz, 3H).

Cleavage of the oxazolidinons: Evans *et. al.*, J. Am. Chem. Soc. 1982, 104, 1737-1739.

Deprotection of the phthalimids: using hydrazine/EtOH at 20°C: Sasaki, T. *et. al.*, J. Org. Chem. **1978**, *43*, 2320; Khan, M. N. et. al., J. Org. Chem. **1995**, *60*, 4536.

According to the herein disclosed synthetic procedures also the following tubulysin derivatives where synthesized:



The following residues where used:

m = 0, 1, 2, 3;

R^1 = methyl, ethyl;

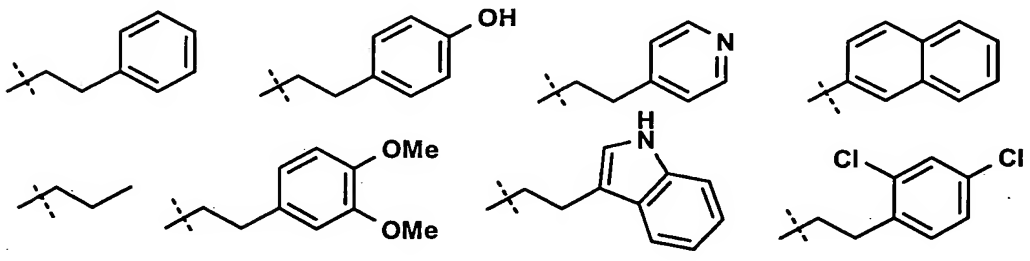
R^6 = isopropyl, isobutyl, ethyl, cyclopropyl, CH_2-cyclopropyl, $CH(CH_3)CH_2CH_3$;

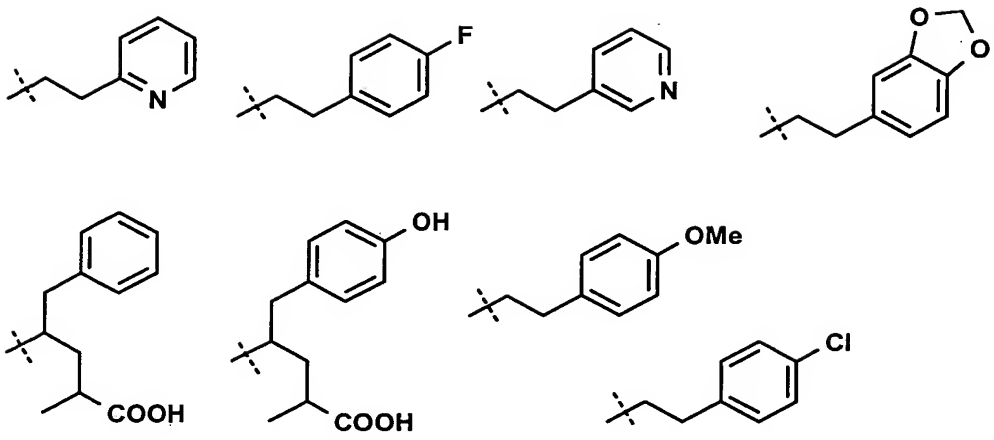
R^9 = isopropyl, trifluormethyl, chlormethyl, isobutyl, ethyl, cyclopropyl, CH_2-cyclopropyl, $CH(CH_3)CH_2CH_3$, cyclopentyl, cyclohexyl;

R^{17} = methyl, ethyl, propyl, isopropyl, butyl, isobutyl, $CH=C(CH_3)$, cyclopropyl, cyclobutyl, cyclohexyl;

R^{20} = methyl, ethyl, propyl, isopropyl, phenyl;

R^{19} =




N
F
N
O
O
COOH
OH
COOH
OMe
Cl